

Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (original) A process for obtaining a population of cells enriched in viable human liver cells, including hepatic stem/progenitor cells, comprising:
 - (a) digesting a whole human liver or resection thereof with a proteolytic enzyme preparation to provide a digested whole human liver or resection thereof;
 - (b) dissociating the digested whole human liver or resection thereof to provide a suspension of cells;
 - (c) adjusting the density of the medium in which the cells are suspended whereby at least two bands of cells separated by a density barrier are obtained upon centrifugation, at least one band of the at least two bands being of a lower density than another band of the at least two bands; and
 - (d) collecting the at least one band of lower density to obtain a population of cells enriched in viable human liver cells, including hepatic stem/progenitor cells.
2. (original) The process of claim 1 in which the population of cells enriched in viable human liver cells further includes functional hepatocytes.
3. (original) The process of claim 1 in which the population of cells enriched in viable human liver cells further includes functional biliary cells.
4. (original) The process of claim 1 in which the population of cells enriched in viable human liver cells further includes functional hemopoietic cells.
5. (currently amended) The process of claim 1 in which step (a) includes:
 - (e) perfusing the whole human liver or resection thereof with a chelation buffer at approximately 37 °C for approximately 15 minutes;

(f) digesting the whole human liver or resection thereof with an enzyme preparation comprising collagenase and at least one other proteolytic enzyme at approximately 37°C for no longer than about 30 minutes to provide a digested liver; and

(g) ~~perfusing the digested liver with collection buffer having a temperature of 4-15°C.~~

6. (original) The process of claim 5 in which the enzyme preparation includes at least one neutral protease.

7. (original) The process of claim 5 in which the enzyme preparation includes elastase.

8. (currently amended) The process of claim 5 in which the enzyme preparation comprises both collagenase and neutral protease LIBERASE™.

9. (original) The process of claim 1 in which said dissociation includes mechanical dissociation.

10. (original) The process of claim 9 in which said dissociation includes mechanical dissociation by cutting, raking, combing, or grating the liver.

11. (currently amended) The process of claim 1 in which step (c) includes at least one of:

- (h) filtering the cell suspension to remove debris and cell aggregates;
- (i) collecting the resulting filtered cell suspension in a first bag;
- (j) optionally determining a concentration of cells in the filtered cell suspension;
- (k) adjusting, if desired, the concentration of cells to provide a starting cell suspension;
- (l) mixing an aliquot of the starting cell suspension with an equal volume of 25%

iodixanol solution in a liquid culture medium to provide a mixture; and

(m) subjecting at least a portion of the mixture overlaid with a predetermined volume of the liquid culture medium to centrifugation to obtain at least one band enriched for viable human liver cells.

12. (currently amended) The process of claim 1 in which step (d) includes at least one of:

- (n) collecting the at least one band into a container on ice;
- (o) optionally determining viability and concentration of cells;
- (p) washing the cells by centrifugation and resuspension in a cryopreservation buffer to obtain a final cell suspension;
- (q) subjecting the final cell suspension to controlled rate freezing to provide a frozen cell suspension; and
- (r) storing the frozen cell suspension in a liquid nitrogen freezer.

13. (original) The process of claim 5 in which said collection buffer comprises RPMI 1640 medium with 10% human or bovine serum.

14. (original) The process of claim 11 in which said filtering step includes passing said cell suspension through a filter cartridge.

15. (currently amended) The process of claim 11 in which said liquid culture medium comprises RPMI 1640 medium lacking phenol red.

16. (original) The process of claim 11 in which said centrifugation is carried out for about 15 min at approximately 500 x g.

17. (original) The process of claim 12 in which said container includes a collection

bag.

18. (original) The process of claim 12 in which the cryopreservation buffer comprises a mixture including Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^- , H_2PO_4^- , HCO_3^- , HEPES, lactobionate, sucrose, mannitol, glucose, Dextran-40, adenosine, glutathione, or combinations thereof.

19. (original) The process of claim 18 in which the cryopreservation buffer further comprises serum and dimethylsulfoxide.

20. (original) The process of claim 19 in which the mixture, serum and dimethylsulfoxide are present in a ratio of approximately 80:10:10 v/v/v.

21. (original) The process of claim 19 in which the serum comprises human serum, bovine serum, or a combination thereof.

22. (original) The process of claim 1 in which the density of the medium is adjusted by the use of an aqueous solution of iodixanol or iohexol.

23. (original) The process of claim 22 in which the aqueous solution of iodixanol or iohexol comprises sterile 60% (w/v) iodixanol in water, and equivalent density of iohexol in water, or a combination thereof.

24. (original) The process of claim 1 in which the density of the medium is adjusted by the use of an aqueous solution of a hydrophilic polymer of sucrose.

25. (original) The process of claim 24 in which the aqueous solution of a hydrophilic polymer of sucrose comprises ficoll, ficoll plus diatrizoate with calcium EDTA, or a combination thereof.

26. (original) The process of claim 1 in which the enriched population of cells includes hepatic progenitor/stem cells having a diameter in the range between 9 and 13 microns and which are positive for expression of EP-CAM, CD133, or both.

27. (currently amended) A process for obtaining an enriched population of viable

human liver cells, which population of cells comprises functional hepatocytes and hepatic stem/progenitor cells, comprising:

- (a) providing a whole human liver or resection thereof from neonatal, pediatric, juvenile, adult, or cadaver donor;
- (b) perfusing the whole human liver or resection thereof with a chelation buffer at approximately 37 °C for approximately 15 minute;
- (c) digesting the whole human liver or resection thereof with an enzyme preparation comprising collagenase and elastase at 37 °C for no longer than about 30 minutes to provide a digested liver cell suspension;
- (d) perfusing the digested liver with chilled collection buffer;
- (e) optionally, mechanically dissociating the digested liver the whole liver or resection thereof to provide a cell suspension;
- (f) optionally, passing the cell suspension through a filter cartridge to remove removing debris and cell aggregates;
- (g) collecting the resulting filtered cell suspension in a first bag;
- (h) optionally determining viability and concentration of cells in the filtered cell suspension;
- (i) adjusting the concentration to about 25 million cells per mL to provide a starting cell suspension;
- (j) mixing in a second bag an aliquot (250 mL) of the starting cell suspension with an equal volume of 25% iodixanol (OptiPrep™) solution in RPMI 1640 medium lacking phenol red;
- (k) subjecting (500 mL) of the resulting mixture overlaid with a predetermined

volume (60 mL) of RPMI 1610 culture medium lacking phenol red to centrifugation on a COBE™ 2991 Cell Processor (15 mm at 2000 rpm, ca. 500 x g) to obtain at least two bands of cells separated by a density barrier, at least one band being of a lower density than another band bands at least one band enriched for viable cells; and

- (l) collecting the at least one band of lower density ~~into a third bag on ice;~~
- (m) ~~optionally, determining viability and concentration of cells in the third bag;~~
- (n) ~~washing the cells in the third bag by centrifugation and resuspension in eryopreservation buffer to obtain a final cell suspension;~~
- (o) ~~subjecting the final cell suspension to controlled rate freezing to provide a frozen cell suspension;~~
- (p) ~~storing the frozen cell suspension in a liquid nitrogen freezer.~~

28. (original) The process of claim 27 in which the enriched population of cells is enriched in hepatic progenitor/stem cells having a diameter in the range between about 9 and about 13 microns and which are positive for expression of EP-CAM, CD133, or both.

29-87. (canceled)

88. (new) The process of claim 27 in which the perfusing is carried out with a chelation buffer.

89. (new) The process of claim 27 in which the enzyme preparation comprises collagenase, elastase, or both.

90. (new) The process of claim 27 in which the removing of debris and cell aggregates is carried out by passing the cell suspension through a filter cartridge.

91. (new) The process of claim 27 in which the iodixanol solution is in RPMI 1640 medium.

92. (new) The process of claim 1 in which the density of at least one band of lower density is less than 1.0792.

93. (new) The process of claim 1 in which the density of at least one band of lower density is 1.0607.

94. (new) A method of obtaining an enriched population of viable human liver cells, which population of cells comprises functional hepatocytes and hepatic stem/progenitor cells, comprising:

- (a) providing a whole human liver or resection thereof;
- (b) digesting the whole human liver or resection thereof to provide a suspension of liver cells;
- (c) mixing an aliquot of the suspension of liver cells with a solution of iodixanol;
- (d) centrifuging the resulting mixture to obtain at least one band enriched for viable cells; and
- (e) collecting the at least one band of viable cells.

95. (new) The method according to claim 94 in which the liver is from neonatal, pediatric, juvenile, adult, or cadaver donor.

96. (new) The method of claim 94 in which the digesting is performed with an enzyme preparation comprising collagenase, elastase or a combination thereof.

97. (new) The method of claim 94 in which the solution of iodixanol comprises 25% (w/v) iodixanol in water.

98. (new) The method of claim 94 in which the solution of iodixanol lacks phenol red.

99. (new) The method of claim 94 further comprising overlaying the resulting

mixture of liver cells and solution of iodixanol with a predetermined volume of medium lacking phenol red prior to the centrifuging step.

100. (new) The method of claim 94 in which the centrifuging is performed on a COBE™ 2991 Cell Processor.